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Growth assessment of Water Soluble Fractions of Crude Oil and Some of its Hydrocarbons Fractions Using a Diatom (*Nitzschia palea*).

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ABSTRACT: *Growth assessment of water soluble fractions (WSF) of crude oil and some of its hydrocarbon fractions were carried out using a fresh water microalga (Nitzschia palea). The microalga was grown separately in ratios 1:3 and 1:9 of 10%, 25%, 50%, 75% and 100% of water-soluble fractions (WSF) of Forcados blend crude oil and hydrocarbons (Hexane, Benzene, Toluene and Xylene). Algal growth response was evaluated using spectrophotometer at an absorbance of 745nm. Growth stimulation was recorded by the alga at all concentrations of the various WSFs. Also, microalgal growth stimulation in ratio 1:3 crude oil water ratios were higher than that of 1:9 crude oil water ratios why the reverse was the case in WSFs of the hydrocarbons. Among the hydrocarbons investigated, the order of growth stimulation of the alga is Hexane > Xylene > Benzene > Toluene. Total biomass synthesized in WSF of crude oil fractions was lower than that synthesized by the WSF of crude oil*

KEY WORDS*: Bioassessment, Crude oil, Exponential growth, Hydrocarbons fractions, Nitzschia palea, Water soluble fractions.*

I. INTRODUCTION

Algae are photosynthetic non-vascular plants containing chlorophyll and possessing simple reproductive structures [12 and 13]. Algae provide much of the earth's oxygen demands, serve as the food base for almost all aquatic life, provide food, pharmaceutical, and industrial products for humans. Their importance as bio-indicators of organic pollution has been explored more recently in the management of waste waters [8 and 7]. Since algae are autotrophic plants, they share with the more advanced autotrophic members of the plant kingdom, the capacity to synthesize complex molecules out of carbondioxide and water, using various elements. The continuity of lives of animals thus hangs on this process. Directly or indirectly, land animals depend on land plants for food while aquatic animals rely on aquatic plants as their basic source of chemically bound energy.

The exploration and drilling for oil on land and offshore involves environmental modification. For instance, vegetation is cleared to make way for seismic lines, drilling site are leveled , road are constructed, drilling mud and oil may reach creeks, thus, quantities of petroleum and its derivatives may be released into the environment during exploration, storage, processing and distribution. Despite the economic and social benefits from oil, there is serious environmental degradation through oil spillage. The production and transportation of oil involve many mechanical processes, the continuous efficiency of which may be hard to guarantee and invariably a spillage may occasionally result because of faults at any stage of production and transportation. When there is an oil spill on water, spreading immediately takes place. The gaseous and liquid components evaporate. Some are dissolved in water and some oxidize, and yet some undergo bacterial changes and eventually sink to the bottom by gravity. The water is then contaminated with a gross effect on the aquatic life. As the evaporation of the lower molecular weight components affect aerial life, so the dissolution of the less volatile component with the resultant water-soluble fractions potent to aquatic lives. The harmful effects of oil spill on the environment are many. For instance, oil kills plants and animals in the estuarine zone, it settles on beaches and kills organisms that live there, and it also settles on ocean floor and kills benthic organisms such as corals and crabs. Oil poisons algae, disrupts food chain and decreases the yield of edible crustaceans. Oil coats birds' feathers, impairing their flight and reducing the insulating potential of their feathers, thus making them vulnerable to cold [1]. Oil endangers fish hatcheries in coastal waters and as well contaminates the flesh of commercially valuable fish [9]. The objective of this study is to assess the effect of water-soluble fraction of crude oil and some of its fractions on the growth a diatom.

II. MATERIALS AND METHODS

Test Microalga: The microalga species used in this study was *Nitzschia palea*. This species was preferred for use because much is known about its morphology and physiological response in culture [10].

Isolation of Pure Culture of Microalga : Unialgal culture of the microalga used for this investigation was isolated from a water body collected around Benin metropolis. This was subjected to series of sub culturing in modified Chu 10 artificial growth medium. An aliquot of the water sample collected was taken and used as inoculum to inoculate the growth medium and allowed to grow. Microscopic examination and identification of microalga was made using relevant texts [12 and 8].

Culture Medium : The microalga species was grown in an artificial batch culture prepared according to Chu's modified No 10 medium [5].

Culture Vessels : Nine hundred and fifty ml round bottomed transparent glass bottles were used for both sub culturing and in the main experiment. They were washed thoroughly with detergent and further rinsed with solution of nitric acid and sulphuric acid separately to remove any algal spore present.

Crude Oil Source : The crude oil used for this study was an unweathered Forcados blend. It was collected from a well head at Oginibo in Ughelli south local government area of Delta state.

Preparation of Water Soluble Fractions (WSF) : The WSF was prepared in ratio 1:3 and 1:9 according to the method of [3] and [11] while the stock WSF was diluted with the culture medium serially to give 10%, 25%, 50%, 75% and 100% WSF.

Experiment Design : Various concentrations (0%, 10%, 25%, 50%, 75% and 100%) of water soluble fractions of crude oil were prepared from the growth medium and made into replicate.

Inoculation : Four hundred and fifty ml each of the water-soluble fractions of the above concentrations and ratios were measured separately in the experimental vessels. These were then inoculated seperately using 2mls of *N.palea* with a 5ml syringe. Each experiment was set up in triplicate. The experimental bottles were plug with sizeable cotton wool to limit evaporation and prevent contamination from the environment. Thereafter, 25mls aliquot each was taken from each triplicate for analysis.

Growth measurement: Growth of microalga was assessed using absorbance. Twenty-five mL aliquot were collected every other day for spectrometric analysis at 745nm using HACH DR 2000 [4].

Statistical Analysis : Apart from the calculated means, Standard error of mean, Analysis of variance was also calculated to detect significant difference between the levels of toxic effect of various concentrations of the treatment on the different microalgae. Where there was significant difference, the Duncan multiple tests was carried out. The statistical package used was the SPSS version 15.

III. RESULTS

The result of the bio assessment of the water-soluble fractions of crude oil and its fractions is represented in Fig

1-10.

Fig 1 and 2 shows response of *Nitzschia palea* in different concentrations of WSF of crude oil in ratios (1:3 and

1:9) respectively. Growth stimulation was observed in all concentrations investigated in both ratios of the WSF of crude oil from day 0 to the end of the study. Growth in ratios 1:9 was more exponential than growth in ratio 1:3.

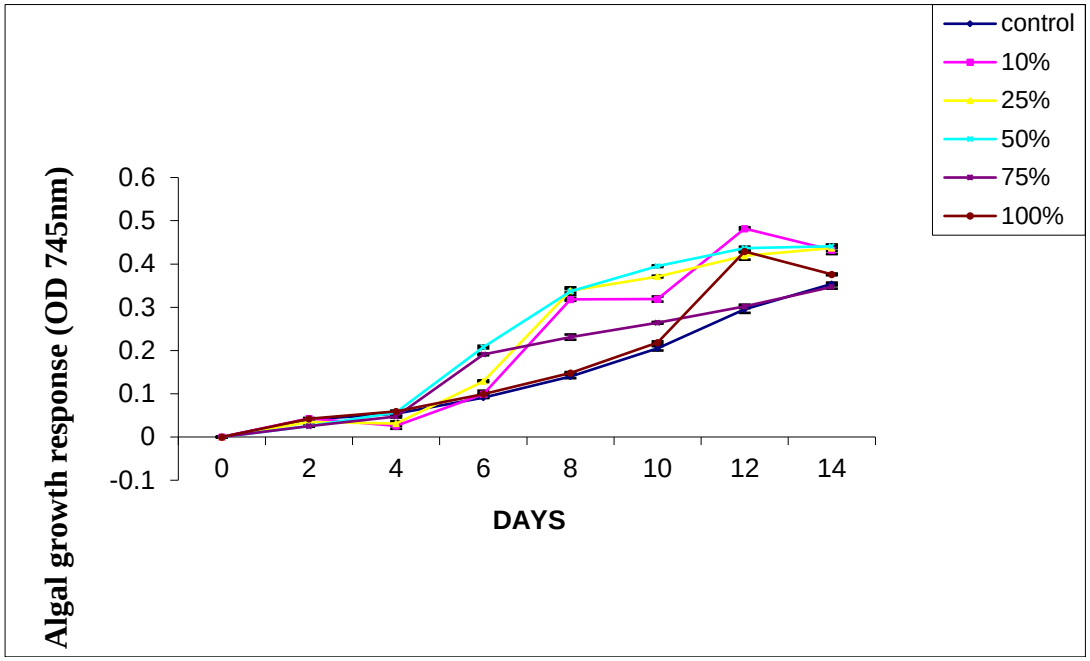

Fig 1: Response of *Nitzschia palea* in different concentrations of WSF of crude oil (1:3)

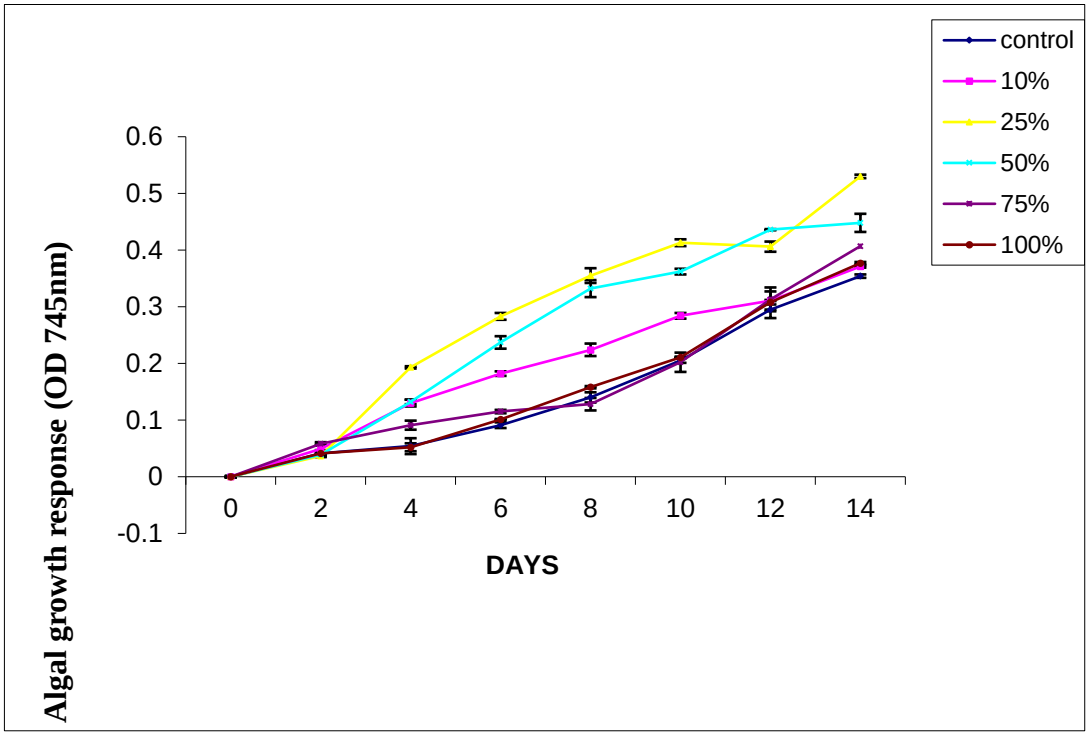

Fig 2: Response of *Nitzschia palea* in different concentrations of WSF of crude oil (1:9)

The growth responses of *Nitzschia palea* in different concentrations of WSF Xylene in ratios (1:3 and 1:9) are shown in Fig 3 and 4. There was a gradual increase in growth in both ratios during the first four days of the study in all concentrations except in 100% concentration where an initial lag phase was observed between day 0 and 2 of the study. This was followed by an exponential increase in growth that lasted till the end of the study in all concentrations.

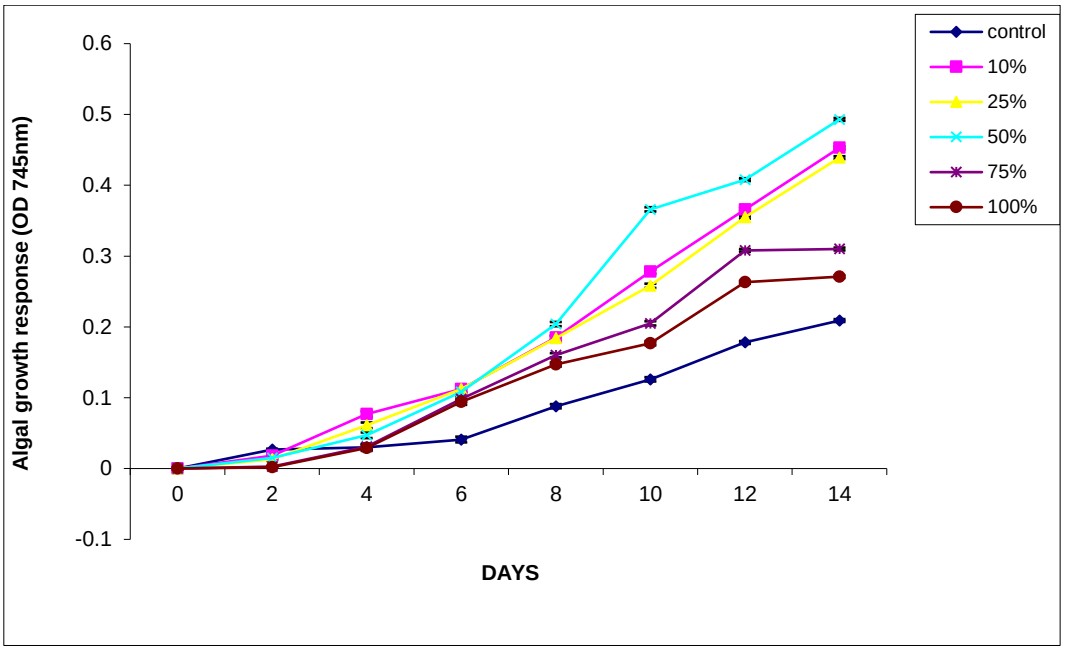

Fig 3: Response of *Nitzschia palea* in different concentrations of WSF Xylene (1:3)

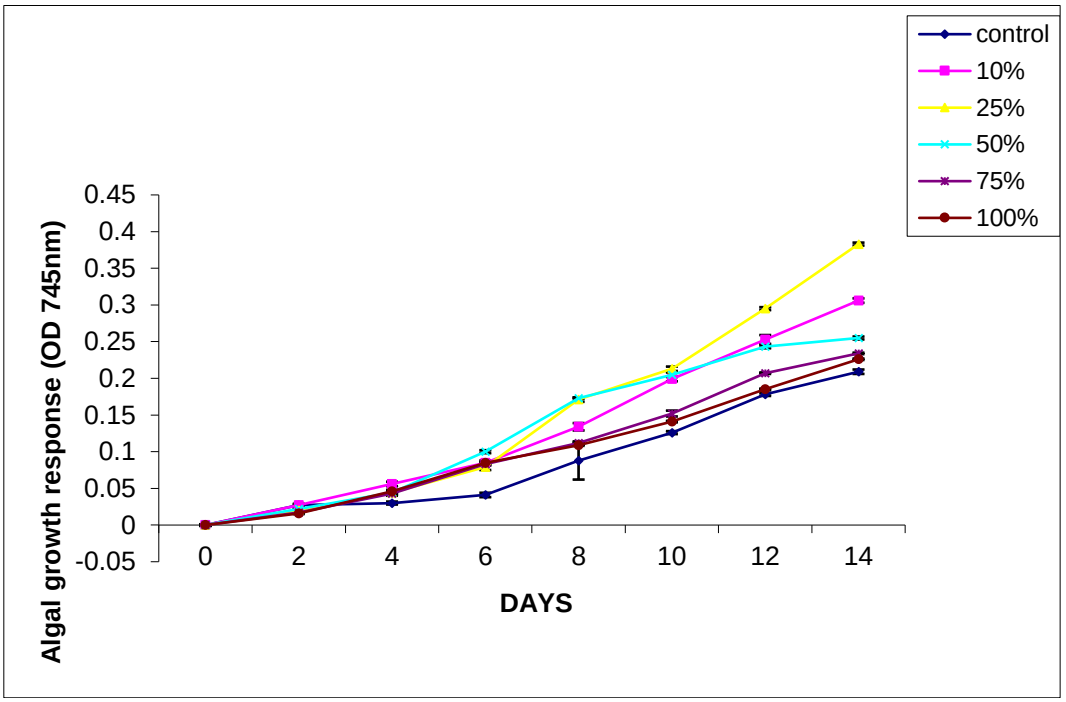

Fig 4: Response of *Nitzschia palea* in different concentrations of WSF Xylene (1:9)

Fig 5 and 6 depicts the response of *Nitzschia palea* in different concentrations of WSF Toluene in ratios (1:3 and 1:9). In the WSF of Toluene, a lag phase of growth was observed in the early age of the culture between day 0 and 4 in all concentrations except in 25% concentration where a gradual growth was recorded (Fig 5). Growth were however exponential after the initial lag phase and was consistent throughout the study in all concentrations. In fig 6, growth was exponential and consistent throughout the study in WSF of ratios 1:9.

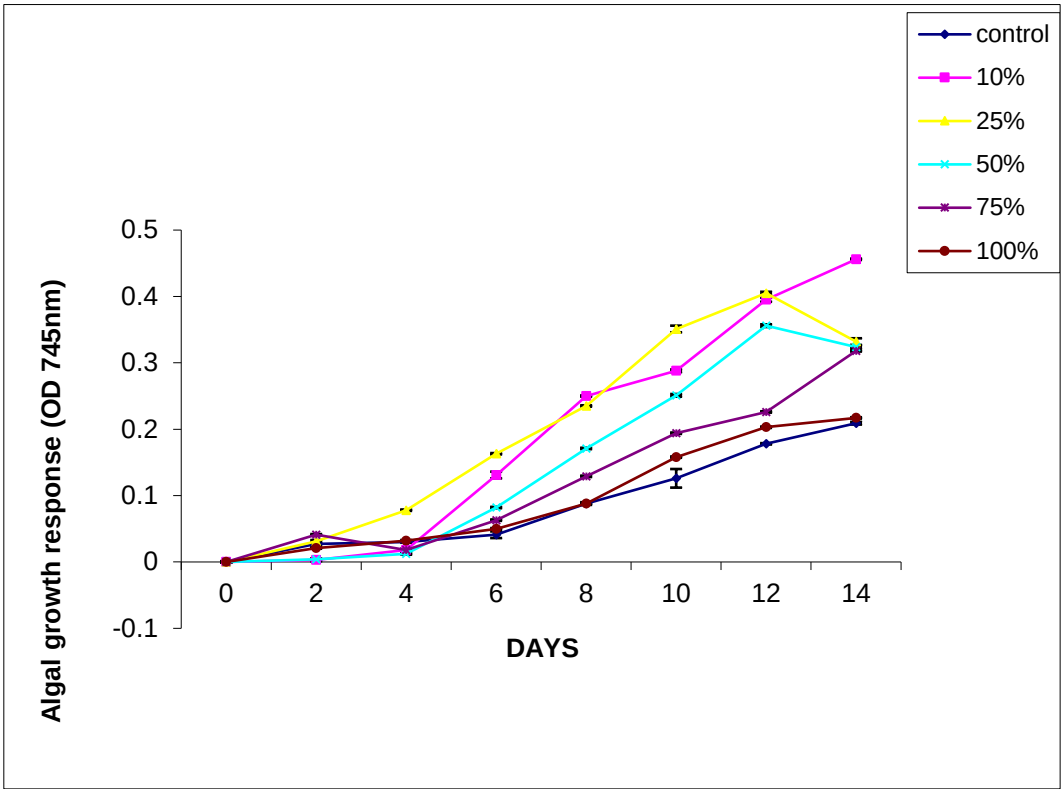

Fig 5: Response of *Nitzschia palea* in different concentrations of WSF Toluene (1:3)

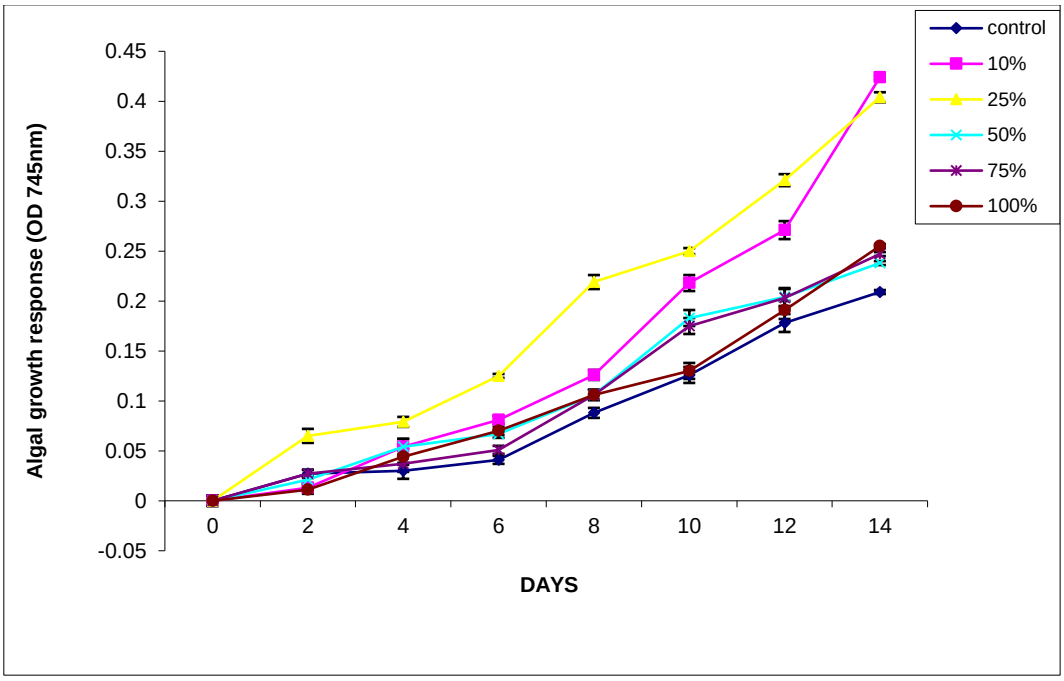

Fig 6: Response of *Nitzschia palea* in different concentrations of WSF Toluene (1:9)

Response of *Nitzschia palea* to different concentrations of WSF Hexane in ratios (1:3 and 1:9) is represented in Fig 7 and 8. Growth suppression was observed from days 0 to 4 (Figs 7) in all concentrations thereafter, a gradual and consistent increase was recorded in growth of the alga to the end of the study. Growth in the control increased gradually throughout the study. In Fig 8 growth was gradual and consistent throughout the study.

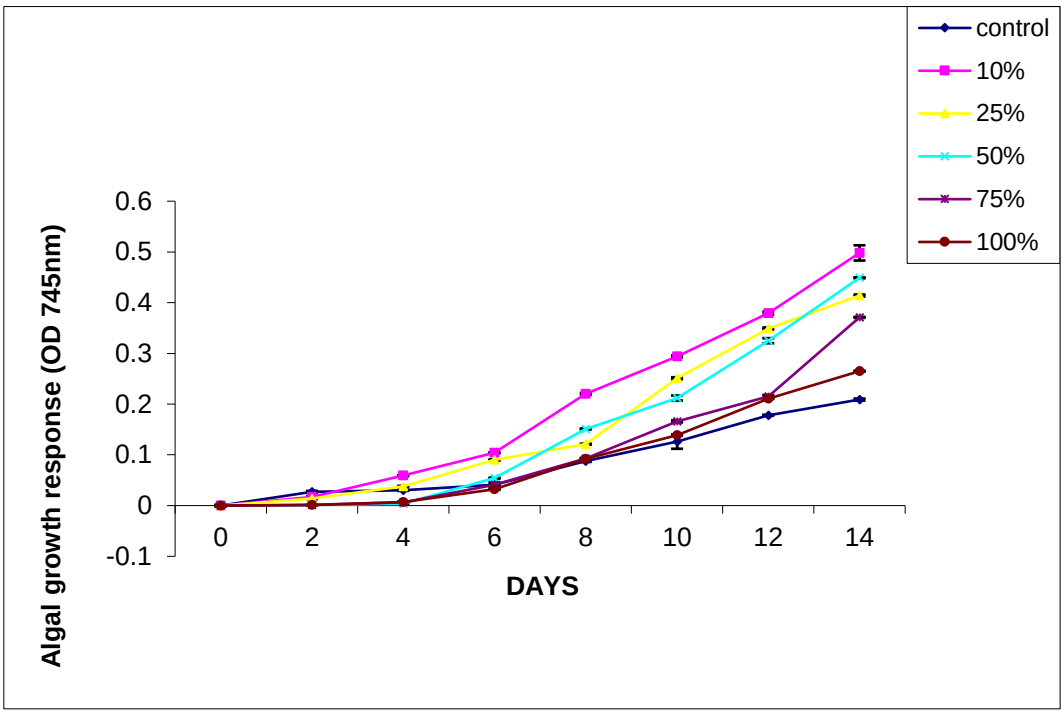

Fig 7: Response of *Nitzschia palea* to different concentrations of WSF Hexane (1:3)

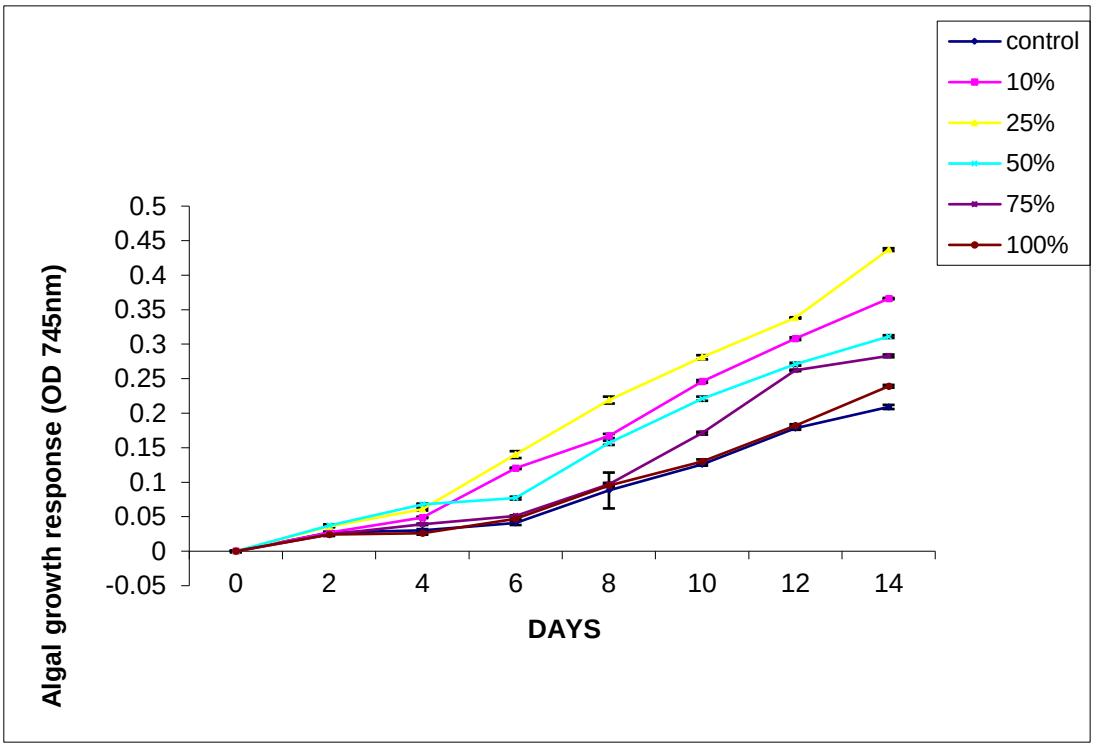

Fig 8: Response of *Nitzschia palea* to different concentrations of WSF Hexane (1:9)

Fig 9 and 10 shows response of *Nitzschia palea* to different concentrations of WSF Benzene in ratios (1:3 and 1:9) respectively. A lag phase of growth was observed in days 0 to day 4 in higher concentrations of the ratio 1:3 (Fig 9) while a gradual increase in growth was observed from day 0 to the end of the study in lower concentrations. However, in ratios 1:9 there was an exponential increase in growth from day 0 to the end of the study.

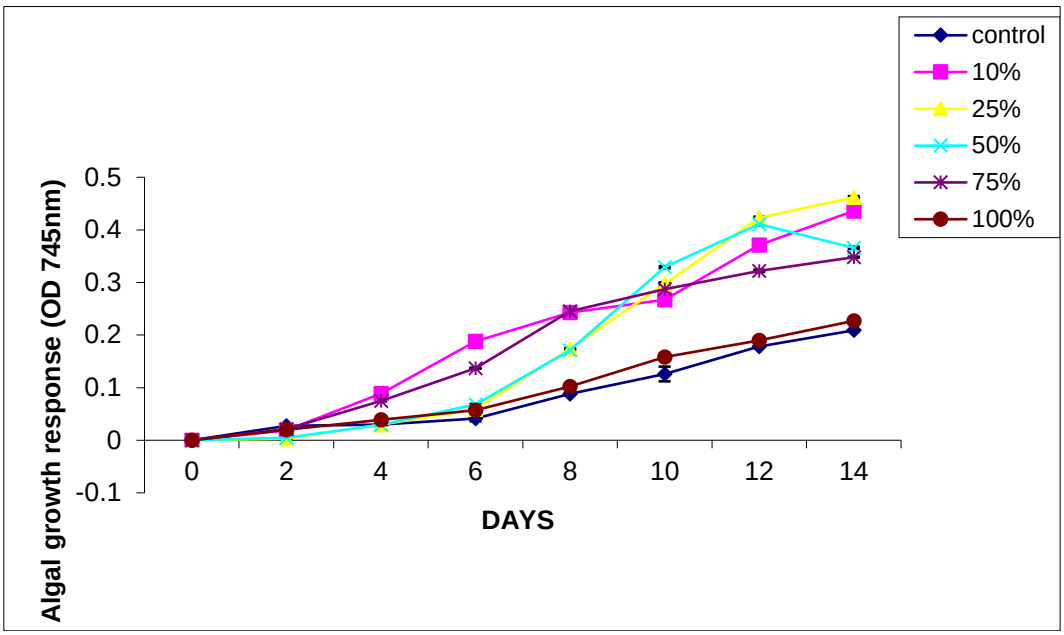

Fig 9: Response of *Nitzschia palea* to different concentrations of WSF Benzene (1:3)

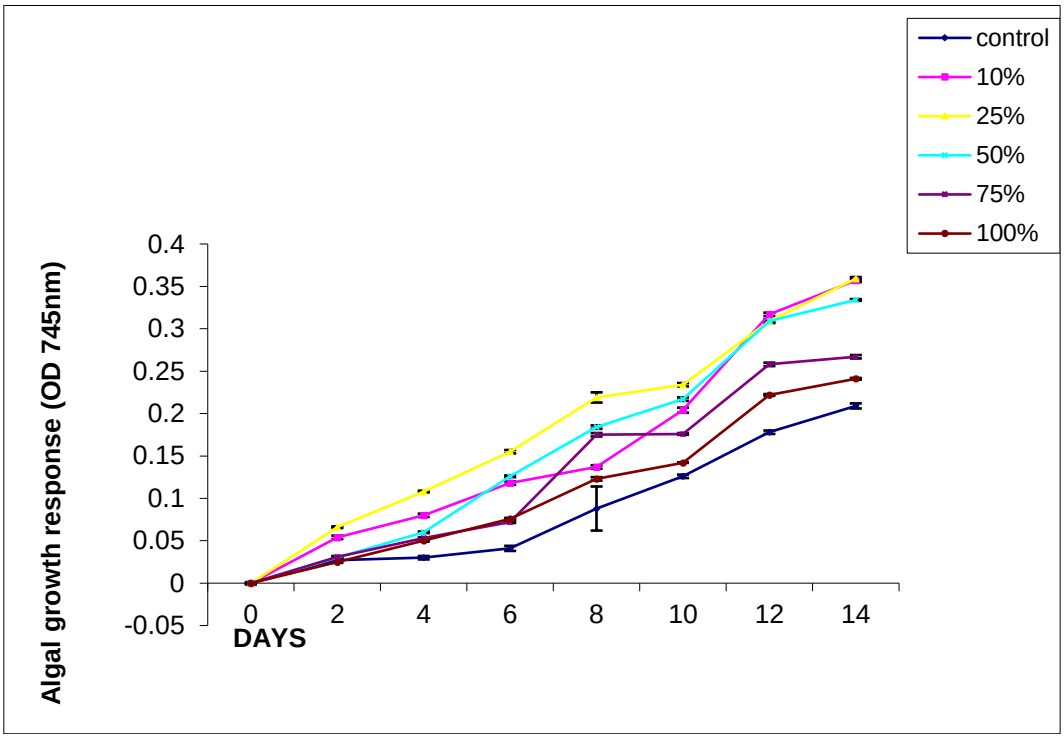

Fig 10: Response of *Nitzschia palea* to different concentrations of WSF Benzene (1:9)

IV. DISCUSSION

Little is known about the physiological responses of microalgae to metabolism of crude oil and its fractions. In general, what is known has been on the use of indigenous hydrocarbon degrading microorganisms that are able to adapt and respond rapidly to various ranges of hydrocarbon contaminations [6]. In this study, the growth response of a diatom in WSFs of crude oil and its hydrocarbon content was investigated the study shows growth stimulation in all WSFs. This shows the ability of the microalga to utilize the contaminant as carbon sources. This finding is similar to that [2] who investigated the response of an algal consortium in diesel; under varying culture conditions

in the laboratory by applying a diesel tolerant sessile fresh water consortium obtain from the vicinity of Powai Lake Mumbai, India. The presence of diesel in batch cultures enhanced the maximum specific growth rate of the algal consortium with decrease in light- dark cycle from 20:4 to 4:20 h. The removal of diesel was found to be highest at Light-dark cycle of 18:6 h with 37.6% degradation over and above controls. In addition to growth in the form of green clumps, white floating biomass was found surrounding the diesel droplets on the surface. This culture predominated at the least Light-dark period of 4:20 h. The author concluded that the floating organisms were able to grow heterotrophically in the dark, utilizing diesel as carbon source and also in the presence of light in a medium devoid of organic carbon source.

The growth suppression observed could be attributed to initial deleterious effects of the WSFs on the microalga while the gradual increase in growth could be due to the ability of the alga to overcome the effects of the contaminant after the initial set back. This finding is similar also to the finding of [11]. The toxic effect of water-soluble fractions (WSF) of fuel oil on a microalga (*Tetraselmis gracilis*) was examined by [11]. On applying different concentrations of WSF, a decrease in cell population was observed. The authors noted that, depending on different physicochemical properties, the hydrocarbons showed different inhibitory effect on growth of the alga. Petrol was reported to be the most toxic and produced inhibitory effect even at lower concentrations. Kerosene did not apparently alter the growth either at lower or higher concentrations. According to the authors, experimental data showed that different oils have different potentials for environmental damage, depending on the types and concentration of soluble and dispersed hydrocarbon present in it.

The growth stimulation observed could be attributed to utilization and mineralization of the components of the crude oil and its fractions. It could also be due to presence of growth promoting substances in the WSF of crude oil other than hydrocarbons which could have stimulated the growth of the microalga. Various authors had reported the responses of microalgae exposed to difference concentrations of hydrocarbons. [11] reported both stimulation and inhibition on growth of microalgae at lower concentration 8% - 10% of WSF of crude oil in their study involving the water-soluble fractions of four different oils on the growth of a microalga. The absence of significant difference at ($p<0.05$) observed in the growth of the alga in lower concentrations and higher concentrations of both ratios when compared with that in the control experiment suggests indifferent effect of the SWFs to the microalga in respective of the contaminant level. Total algal growth stimulation in both ratios were higher in WSF of treatment cultures of crude oil water ratio than WSF of hydrocarbons and control experiment and there was significant difference ($p<0.05$) in growth of the alga in the various concentrations of the treatments (10% - 100%). This could be attributed to differences in dissolution of components of crude oil into water at the different ratios investigated. In this study, TPH content of WSF of ratio 1:3 was higher than that of ratios 1:9. Differences in dissociation of hydrocarbons into solution could also have resulted in WSF of hydrocarbons yielding higher growth stimulation.

V. CONCLUSION

Growth stimulation was recorded by the alga in all concentrations of the various WSFs of both ratios. Also, microalgal growth stimulation in ratio 1:3 crude oil water ratios were higher than that of 1:9 crude oil water ratios why the reverse was the case in WSFs of the crude oil fractions (hydrocarbons). Among all the hydrocarbons investigated, the order of growth stimulation of the alga is Hexane > Xylene > Benzene > Toluene.

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